CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 20-903

MICROBIOLOGY REVIEW(S)

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JUN 1 1998

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA #: 20-903

REVIEWER	:N. Battula
CORRESPONDENCE DATE	:12-03-97
CDER RECEIPT DATE	:12-03-97
REVIEW COMPLETE DATE	:04-27-98

SPONSOR: Schering Corporation
2000 Galloping Hill Road
Kenilworth, NJ 07033

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Anti-HCV

INDICATION: Treatment of chronic hepatitis C infection

DOSAGE FORM: Intron A as lyophilized powder and Ribavirin as capsules

PRODUCT NAMES:

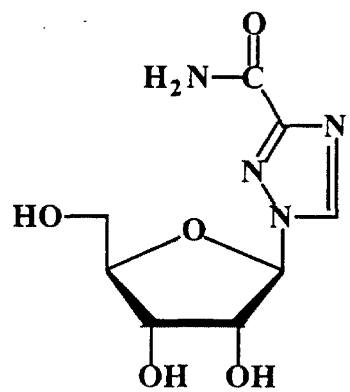
A. PROPRIETARY: (1) Intron A and (2) Rebetol

B. NONPROPRIETARY: (1) Intron alfa-2b and (2) Ribavirin

C. CHEMICAL: (1) H_2N-Cys-Asp-Leu-Pro-Gln-Thr-His-Ser-Leu-Gly-Ser-Arg-Arg-Thr-Leu-Met-Leu-Leu-Ala-Gln-Met-Arg-Arg-Ile-Ser-Leu-Phe-Ser-Cys-Leu-Lys-Asp-Arg-His-Asp-Phe-Gly-Phe-Pro-Gln-Glu-Glu-Phe-Gly-Asn-Gln-Phe-Gln-Lys-Ala-Glu-Thr-Ile-Pro-Val-Leu-His-Glu-Met-Ile-Gln-Gln-Ile-Phe-Asn-Leu-Phe-Ser-Thr-Lys-Asp-Ser-Ser-Ala-Ala-Trp-Asp-Glu-Thr-Leu-Leu-Asp-Lys-Phe-Tyr-Thr-Glu-Leu-Tyr-Gln-Gln-Leu-Asn-Asp-Leu-Glu-Ala-Cys-Val-Ile-Gln-Gly-Val-Gly-Val-Thr-Glu-Thr-Pro-Leu-Met-Lys-Glu-Asp-Ser-Ile-Leu-Ala-Val-Arg-Lys-Tyr-Phe-Gln-Arg-Ile-Thr-Leu-Tyr-Leu-Lys-Glu-Lys-Lys-Tyr-Ser-Pro-Cys-Ala-Trp-Glu-Val-Val-Arg-Ala-Glu-Ile-Met-Arg-Ser-Phe-Ser-Leu-Ser-Thr-Asn-Leu-Gln-Glu-Ser-Leu-Arg-Ser-Lys-Glu-COOH

(2) 1-β-D-1*H*-ribofuranosyl-1,2,4-triazole-3-carboxamide

STRUCTURAL FORMIULA: (2)



SUPPORTING DOCUMENTS:

BACKGROUND: Schering Corporation submitted this NDA in support of their application for use of a two drug combination consisting of Intron A (Interferon-alfa-2b. recombinant) plus Ribavirin vs. Intron A alone, for the treatment of chronic hepatitis C in individuals who have relapsed following a course of interferon-therapy. Intron A was previously approved for the treatment of hepatitis C infection and Ribavirin was approved for respiratory syncytial virus infections.

Ribavirin was subsequently licensed to Schering Corporation for further development in the treatment of HCV infection.

The sponsor, Schering Corporation, requested a priority review of this application. The clinical portion of this application was submitted as a computer assisted new drug application. The requested indication is based on changes in virologic (HCV RNA), hepatohistopathologic (Knodell Score) and biochemical (alanine aminotransferase) surrogate markers scored as end-of-treatment response at 24 weeks of therapy and sustained response at 12 and 24 weeks of off-therapy follow-up.

In the microbiology portion of the submission the sponsor has not provided information on the mechanism of hepatitis C virus inhibition by interferon and/or ribavirin or the method for the determination of HCV RNA by Reverse Transcription-Polymerase Chain Reaction (RT-PCR). However, after repeated oral and written requests, the sponsor submitted scant information related to the RT-PCR. The microbiology review here deals with 1. Virology of hepatitis C virus, 2. An analysis of the experimental RT-PCR for determination of HCV RNA, 3. Postulated mechanism of action of interferons and ribavirin and 4. HCV RNA determination in clinical studies.

1. HCV VIROLOGY: Hepatitis C virus has a short history. Until 1975 only two hepatitis viruses, hepatitis A virus and hepatitis B virus were known. A proposal for the potential existence of non-A and non-B hepatitis viruses was put forth in 1975 when it was discovered that some hepatitis patients lacked serological reactivity against hepatitis A and B viruses. The presence of non-A and non-B virus i.e., HCV, was confirmed in 1989 when the cDNA of HCV genome was cloned, characterized and a diagnostic test developed. Lack of permissive cell culture systems for in vitro cultivation of hepatitis C virus hampered studies on the replication and pathology of the virus. However, the use of molecular biology and biotechnology lead to a tremendous progress in HCV virology including its diagnosis. To date six hepatitis viruses, Hepatitis A, B, C, D , E, and G have been recognized and characterized.

<u>HCV structure and genotypes</u>: Hepatitis C viruses are a family of small (50nm) spherical enveloped viruses with single stranded linear RNA genome of positive polarity and hence the viral RNA can serve as a message for synthesis of viral proteins. The size of HCV genome is approximately 9,600 nucleotides (nt) consisting of a relatively long (332 to 341 nt) 5'-NTR (non-translated region), a large open reading frame encoding a polyprotein of >3,000 amino acids (~9033 nt) and a relatively short (45 nt.) 3'-NTR followed by homopolymeric tail of poly A or U residues.

The precursor polyprotein of HCV is proteolytically processed by the combined action of viral and cellular proteases to generate ten mature virion proteins. Three structural proteins; C, M and E (core, matrix and structural protein, respectively), localized at the 5'-end are involved in forming the viral particles and the seven nonstructural proteins NS1, NS2a, NS2b, NS3, NS4, NS4b and NS5, localized to the 3'-end are thought to be involved in the replication of the viral genome. The 5'-NTR of HCV is relatively the most conserved portion among all HCV isolates and as such it is often used for determination of HCV RNA by RT-PCR.

HCV being an RNA virus is highly mutable and accordingly, in infected individuals the HCV complex consists of a continuum of genetic variants. The extent of genetic variation in HCV-infected individuals is suggested by recent kinetic studies[1] which indicate a half-life of 8 hours for HCV with a turnover rate of $\geq 3.7 \times 10^{11}$ virions /day to maintain a serum virion concentration of 10^7 virions/ml. The high rate of replication and turnover of HCV at $\geq 3.7 \times 10^{11}$ virions/day far exceeds that of the HIV turnover rate of 10^8 to 10^9 virions/day. This extraordinarily large number of replication cycles in vivo results in evolution of highly divergent quasispecies of HCV and thus could challenge even the most promising antiviral agent(s).

Based on phylogenetic analysis of the coding regions, HCV has been classified into at least six distinct genotypes and the number of genotypes and their sub-types has been expanding as more isolates from different parts of the world are discovered and sequenced. HCV genotypes 1, 2 and 3 show a relatively broad geographic distribution, and 4, 5 and 6 are more restricted. Recent studies suggest that genotypes influence the efficiency of transmission and response to therapy. These studies were mostly based on assay procedures that detect and quantify HCV RNA genotypes with varying efficiencies[2] and lack of genotype specific quantitation makes the results unreliable.

Detection and diagnosis of HCV: To date there are no in vitro models to support the growth of HCV. Molecular biology techniques lead to the discovery of HCV in 1989 and biotechnology lead to our current ability to detect, diagnose and prevent HCV infection. There are two general categories of tests that define HCV infection: (1) Serological assays that can detect antibodies to HCV and (2) Molecular tests that claim to detect, quantify and characterize HCV RNA genomes.

Serological tests: Table 1 shows the progressive development of three generations of anti-HCV enzyme immunoassays (EIA) for the detection of HCV infection, their relative sensitivities and their positive predictive value. The first serological screening assay for detecting anti-HCV contained a single HCV antigen produced by recombinant DNA techniques. Although this assay was very useful in identifying individuals with serological evidence of HCV infection, it lacked optimal sensitivity and specificity. The EIA-1 test was subsequently replaced (Table 1) with EIA-2 and EIA-3 that contained additional viral epitopes which lead to substantial improvements in sensitivity and specificity of HCV antibody detection relative to the EIA-1. In addition, the latter EIA tests shortened the average 'window period' i.e., the time interval between primary HCV infection to seroconver-

[1] Lam, N. et al., Hepatology (1977) **26**: 226-231

[2] Hawkins, A. et al., J Clin Microbiol (1977) **35**: 187-192

sion. so that the mean time to detection was reduced to 10 weeks with EIA-2 compared to 16 weeks with EIA-1.

Table 1. Sensitivity and positive predictive value of EIA for anti-HCA*

			Positive Predictive Value (%)	
Year[1]	Assay	Sensitivity	Low prevalence	High prevalence
1990	EIA-1	70-80	30-50	70-85
1992	EIA-2	92-95	50-61	88-95
1997	EIA-3	97	25	Not done

* Most contents of the table are from [3]
[1] Year of assay availability

The anti-HCV EIA tests were primarily designed to optimize the sensitivity of detection, thereby preventing transmission of HCV infection from blood and blood products and as such EIA tests were not optimized for exquisite specificity. Often these tests also showed false positivity in some individuals who had no other laboratory or clinical evidence of infection. To resolve false positivity of EIA tests, other supplementary confirmatory tests such as the recombinant immunoblot assays (RIBA), which were technically more demanding but conferring greater specificity compared to EIA, were developed and these additional tests have been used in some cases to aid in the diagnosis of HCV infections. Immunodetection methods including RIBA test are not always indicative of ongoing HCV infection because HCV recovered patients may remain HCV antibody positive for many years. Therefore, molecular tests such as RT-PCR diagnostic of ongoing HCV infection need to be used for confirmation of infection.

Molecular tests: Molecular tests directly detect HCV infection and are thus independent of seroconversion. These tests in addition to being confirmatory of HCV infection also detect infection early in the 'window period'. Available molecular assays for HCV can be divided into two types: 1. qualitative tests that detect the presence or absence of HCV RNA in test samples and 2. quantitative tests or viral load tests that determine quantity of HCV RNA in test samples. The molecular tests are based on two approaches: A. signal amplification based methods such as the Chiron b-DNA assay and B. sequence amplification methods such as the RT-PCR. Thus far none of the molecular tests have received approval by the FDA.

[3] Gretch, D. R. Hepatology (1977) 26: 43S-47S

Qualitative assays: In the published literature many "home-brew" RT-PCR based qualitative assays have been described. These tests reported different degrees of sensitivity for the detection of HCV with the most sensitive tests claiming a capability of detecting 100 copies /ml. In general, the RT-PCR assays have played an important role in confirmation of HCV diagnosis and also in assessing antiviral response to therapies such as interferons. However, several attempts to standardize these assays showed lack of uniformity. For example, in a large international collaborative study[4], 86 labs submitted 136 data forms on a panel of coded sera. In these data sets only 16% of the labs scored perfectly on the comprehensive standardized test panel, 55% reported false positive and/or false negative results and 29% missed weak positive sample. Thus, lack of rigorous quality control and proficiency testing of these "home-brew" assays render them often unreliable for use in clinical testing.

Quantitative tests: Two different types of techniques namely, ave been used to determine HCV RNA concentration in infected individuals. Many labs have described their "home-brew" quantitative RT-PCR tests. ; perhaps the only quantitative (not FDA approved) RT-PCR assay available in a kit format. In general quantitative RT-PCR methods have high analytical sensitivity and the Monitor assay is claimed to measure HCV RNA at levels of 1000 copies/ml. The RT-PCR assays for the determination of HCV RNA have high variability and limited dynamic range (1 million copies/ml) and there is limited published experience with these methods. In the clinical studies conducted for this NDA the sponsor used the services of a testing laboratory for determination of HCV RNA in patient sera by an in-house RT-PCR procedure.

In the b-DNA signal amplification procedure HCV RNA in plasma or serum is captured in microtiter wells by hybridization to oligonucleotide probes complementary to the conserved 5'-NTR and the 5' third of the core region of the HCV genome. The amount of RNA captured is quantified by chemiluminescent signal amplification and the quantity is expressed as genome Eq/ml. This method is standardized and there is published experience in clinical studies. However, the hybridization efficiencies varies[2] among HCV genotypes and refinements on oligonucleotide probe became necessary to increase the sensitivity and specificity of the HCV RNA in clinical specimens. Moreover, the b-DNA-1 assay and the b-DNA-2 assay have low analytical sensitivity with a lower limit of quantification of 350,000 and 200,000 RNA equivalents/ml, respectively, and therefore RT-PCR is necessary on b-DNA negative specimens to rule out low-level of viremia.

[4] Damen, M. et al., J. Virol. Meth. (1996) **58**: 175-185

[5] Detmer, J. et al., J. Clin. Microbiol. (1996) **34**: 901-907

[6] Nolte, F. S. J. Clin. Microbiol. (1995) **33**: 1775-1778

In the clinical studies of this NDA the sponsor determined HCV RNA copy number by RT-PCR. In the estimation of HCV-RNA load the RNA copy number used as a reference standard was calculated by the b-DNA assay which expresses RNA as equivalents/ml. The sponsor pooled plasma samples from HCV positive individuals and the viral RNA equivalents/ml of plasma, as determined by b-DNA assay, was used as reference standard for HCV RNA copy number. HCV RNA in clinical trial patient **sera** was determined against the **plasma** control to calculate HCV RNA. A variety of inconsistent criteria renders this unapproved b-DNA assay inadequate as gold standard for calculating RNA copy number. At best this assay may represents a qualitative procedure for estimating HCV RNA in patient serum samples.

HCV genotyping: Published evidence suggests that HCV genotype may be an independent predictor of response to therapy and therefore genotyping of HCV has become an important aspect of clinical trials. Tests for HCV genotyping include:

2. **DETERMINATION OF HCV RNA BY RT-PCR**: The sponsor of this NDA, Schering Corporation,

HCV RNA was determined by coupled reverse transcription and polymerase chain reaction (RT-PCR). The RT-PCR is an experimental assay developed in-house by NGI and was exclusively used by NGI at their facilities. From the initial submission of the IND in 1996, FDA requested the sponsor (both orally and in writing) to provide details of the assay protocols and the performance characteristics of their assay for FDA evaluation. Written requests transmitted to the sponsor are attached to this review as Appendix 1 and Appendix 2. In July, 1997, FDA submitted a list of specific questions (see Appendix 1) regarding the sponsor's RT-PCR, and in response the sponsor in October, 1997, provided very general information on their assay without addressing the questions by data. They made summary statements of their protocols, what they did and the conclusions drawn without providing raw data. The sponsor's RT-PCR procedure essentially involves the following steps:

[2] Simmonds, P. et al., J. Gen. Virol. (1993) **74**: 661-668

[3] Stuyver, L. et. al., J. Gen. Virol. (1993) **74**: 1093-1102

[4] Okamoto, H. et al., J. Gen. Virol. (1993) **73**: 673-679

1. Extraction of HCV RNA from 0.1 ml of serum (no controls included for estimation of RNA recovery)

2. Synthesis of complementary DNA (cDNA) to HCV RNA by using an enormous excess of random hexamers as primers, and M-MuLV reverse transcriptase

3. Amplification of the cDNA by PCR using primers specific to the 5'-NTR. Cycling conditions: polymerization at 72°C, denaturation at 96°C and annealing at 55°C with 60 second duration for each step (the cycling temps and duration stated vary in different volumes of the NDA submission)

4. Electrophoretic separation of PCR products on agarose gels and transfer of the DNA fragments to nitrocellulose membranes

5. Hybridization of DNA fragments on NC with digoxigenin labeled probes and reacting the digoxigenin with anti-digoxigenin antibodies conjugated to alkaline phosphatase

6. Colorimetric detection of the antibody bound DNA after reaction of the alkaline phosphatase with a chromogenic substrate

Review comments on the RT-PCR protocol: There are numerous deficiencies in this experimental RT-PCR procedure and for brevity the comments are limited to a few aspects.

A) Reverse transcription was not optimized to obtain the desired minimum length of ≥257 nucleotide long cDNA. In cDNA synthesis with 300 ng of random hexanucleotide primers and 10 HCV RNA template molecules (at claimed sensitivity of the assay), the ratio of template to primer would be $1{:}>10^{13}$. With such an excess of primer, copying of the highly stable 5'-NTR at 40°C is likely to yield smaller than desired size DNA for PCR amplification. There is no indication of the length of the cDNA products formed or the need for such template:primer ratio. In such studies of cDNA synthesis it is conventional to use gene specific anchored primers to obtain a product of expected size from a defined sequence.

B) The sub-sequence of HCV RNA selected for amplification is the 5'-NTR. The 5'-NTR is the most conserved sequence in HCV, yet it has only about 85% identity among different genotypes and is also of variable length due to deletions and substitutions[10]. The long 133 nucleotide probe used hybridizes to different length PCR products to score as

[10] Lemon, S. M. and Honda, M. Seminars in Virol. (1997) 8: 274-288

positive in the assay. Furthermore, the sponsor has not indicated as to how several of the post-amplification steps that eventually lead to the detection of the DNA were optimized.

C) Assay controls were inadequate or were not included. For example, in the standard copy number control used as reference, the copy number of HCV RNA was determined using b-DNA assay which by itself is an experimental procedure with a lower limit of quantification of 200,000 RNA equivalents/ml. In order to lend support and perspective to arrive at a defined copy number for use as reference it is conventional to use more than one method.

The summary comment on this experimental RT-PCR assay is that it is a qualitative, in house assay with an undefinable number for the lower limit of detection of HCV RNA copies in serum samples.

3. MECHANISM OF ACTION: Neither ribavirin nor Intron A have well recognized targets on HCV to effect inhibition of the virus replication. However, these substances have been in use for other indications and multiple hypotheses have been extended to explain their effects. A brief summary of the proposed modes of action is presented.

Ribavirin: Ribavirin is a 'guanosine nucleoside analogue' which undergoes biotransformation into its mono. di and triphosphate forms in the body. These metabolic forms interfere either directly or indirectly with the nucleic acid biosynthesis of body cells as well as with infectious agents that infect or invade cells. Ribavirin monophosphate, for example, is a well recognized inhibitor of inosine monophosphate (IMP) dehydrogenase which is required for the synthesis of guanylate. The primary effect of IMP dehydrogenase is a reduction in intracellular guanylate nucleotide pools including GTP and dGTP, precursors in nucleic acid biosynthesis. Reduced supply of these precursors in the presence of ribavirin limits the rate of nucleic acid biosynthesis, a consequence that affects both cells and the infecting viruses. Furthermore, dGTP and/or GTP play critical roles in essential cellular signaling and alterations in levels in these molecules may adversely affect cellular metabolic pathways.

An additional recognized effect of ribavirin is its interference with mRNA 'cap' structure formation which is essential for maximal translational efficiency of both cellular and viral mRNAs. The mRNA capping involves the addition of guanosine phosphate on the 5'-ends of mRNAs and subsequent methylation of the guanylated site. Ribavirin inhibits the capping of mRNA either by decreasing guanylate pools or by direct interference with methyl transferases required for the capping process. The positive stranded HCV RNA which naturally lacks 5'-cap structure is translationally competent and initiates protein synthesis by an alternate mechanism, through an internal ribosomal entry site. Therefore, ribavirin may not inhibit HCV by this mode of action i.e., through 'cap structure' mediated translation process. It is interesting to note that several clinical studies[11,12] showed a lack of inhibitory effect of ribavirin on HCV viral load or improvements in hepatic histopathology. On the contrary ribavirin decreases levels of ALT and hemoglobin which are translated from capped mRNAs.

The precise molecular mechanism of action of ribavirin on cells and infectious agents is unknown. It is believed that the drug acts in a complex, multi-site fashion[13]. Unlike classical nucleoside analogues which inhibit nucleic acid biosynthesis by their incorporation and chain termination, ribavirin does not appear to incorporate into nucleic acids and thus its mechanism of action appears to be different from that of the classical nucleoside analogues.

Interferon alfa-2b, recombinant: Interferon alfa-2b is a water soluble protein of 165 amino acids with a molecular mass of 19,271 daltons. The protein is recombinant DNA product expressed in *E.coli* from a plasmid bearing cloned interferon alfa-2b DNA sequence derived from human leukocytes.

Interferons are biological response modifiers. They exhibit redundant functions with overlapping activities on a range of responding cells with their effects varying depending on the tissue. Interferons both up and down regulate gene expression, exhibit both beneficial and detrimental effects and play roles in both physiology and pathology. Genetic and biochemical evidence suggests that the diverse functions of interferons include antiproliferative, antiviral, immunomodulatory and central nervous system effects[14]. Current evidence supports that interferons exert their cellular activities by binding to specific membrane receptors on the cell surface. Upon binding to the cell membrane, interferons initiate a complex sequence of intracellular events through signal transduction pathways[15] that lead to the expression of numerous interferon induced gene products. However, the specific mechanism by which interferon exerts anti-HCV activity is unknown.

11 Di Bisceglie, A. M. Ann. Intern. Med. (1995) **123**: 897-903

12 Dusheiko, G. J. Hepatol. (1996) **25**: 591-598

13 Patterson, J. L. and Fernandez-Larsson, R. Rev. Infect. Dis. (1990) **20**: 1139-1146

14 Gutterman, J. A. Proc. Natl. Acad. Sci. (1994) **91**: 1198-1205

15 Ransohoff, R. M. N. Engl. J. Med. (1998) **338**: 616-618

The specific mechanism of inhibition by ribavirin or Intron A and/or enhancement of inhibition by the combination use of ribavirin and Interferon alfa-2b, recombinant is unknown.

4. HCV RNA DETERMINATION IN CLINICAL STUDIES: The sponsor conducted two similar Phase 3 clinical studies: 1. C95-144, a U.S. trial (n=153), and 2. C95-145, an international trial (n=192). These studies were double blind, placebo-controlled, randomized, parallel-group, multi-center trials, comparing interferon monotherapy with interferon plus ribavirin combination therapy, in patients with chronic hepatitis C who had relapsed following a previous response to a course of interferon. In the two studies HCV-infected individuals were equally randomized to receive either: Interferon 3MU TIW for 24 weeks or Interferon 3MU TIW + Ribavirin, 1000-1200 mg/day (depending on weight) for 24 weeks followed by 24 weeks of off-therapy follow-up.

Virologic criteria for enrollment into these studies was serum HCV RNA positivity as determined by an experimental. in-house, RT-PCR assay. With regard to virology the enrolled patients were stratified by two variables: 1. baseline RNA level, into those bearing $\leq 2x10^6$ RNA copies/ml or $>2x10^6$ RNA copies/ml and 2. HCV genotype. into those bearing the genotype-1 (which included genotype-1 plus mixed genotype) and those bearing other than the genotype-1.

Serum HCV RNA was evaluated at weeks 4, 12. 24 during therapy and at weeks 12 and 24 following the end of therapy. One of the efficacy endpoints was sustained virological response. A virologic responder was defined as a subject whose HCV RNA was below the assay limit of detection at the time point tested. A sustained virologic responder was defined by the sponsor as a subject whose HCV RNA falls below the assay limit of detection at end of 24 weeks of follow-up. FDA modified the sponsor's definition of sustained responders to include those that responded at the end of treatment period of 24 weeks, and at 12 and 24 weeks of post-therapy follow-up. If HCV RNA was detectable by the experimental RT-PCR at any of the 3 time points, then the subject was considered as a nonresponder. In these studies the sponsor also evaluated the response rates on the basis of genotypes and viral load.

In the determination of HCV RNA in patient sera an experimental in-house RT-PCR assay was used. The sponsor indicated that their RT-PCR is a dichotomous assay in which the assay was qualitative when the RNA copy number was below 10^4 RNA copies/ml of serum and quantitative assay at and above 10^4 RNA copies/ml of serum. The sponsor claimed that the lower limit of detection of the qualitative assay was 100 copies of RNA /ml and the linear range for the quantitative assay was 10^4 to 10^6 copies of RNA/ ml of serum. FDA evaluation of their assay showed that the procedure in its entirety was qualitative by design and that there were numerous deficiencies in the protocol to support a claim that this was a quantitative method. Right from the submission of the IND in 1996 till now the sponsor was requested to provide details of the assay protocol and its performance characteristics. The scant information that the sponsor recently provided was inadequate to suggest that it is a quantitative procedure.

With regard to the assay's lower limit of detection it has been difficult to project a specific number because of multiple deficiencies such as the uncertainity of reference copy number control, lack of other controls to evaluate the efficiency of RNA extraction, reverse transcription, DNA amplification and DNA detection. Therefore, for the purpose of this analysis it is simply stated as "lower limit of detection of the assay". The lower limit of detection was further complicated due to the use of different matrices; **plasma** for HCV RNA as copy number as reference control and **serum** as test sample to determine HCV RNA in patients. The RNA equivalents/ml estimated from b-DNA assay was considered synonymous with the RNA copy number/ml estimated by the experimental RT-PCR assay. Additionally published evidence suggests that plasma is a better matrix for preserving RNA viruses than serum[16] and the RNA copy number in plasma by RT-PCR score at higher levels than in serum[17].

Genotyping of HCV was carried out by dot blot hybridization analysis of 5'-nontranslated region of the HCV RNA. The assay has been reported to detect all of the six HCV genotypes and their subtypes. The method is more of a screening procedure and is generally considered acceptable for genotyping. However, the gold standard for genotyping is sequence determination of the HCV RNA. The sequencing procedure for genotyping at this time is not practical for multiple samples and as such it was not used in these studies.

Prior clinical studies suggested that both HCV genotype and baseline viral load were useful in predicting a sustained treatment response to interferon monotherapy[18]. To assess this notion the sponsor stratified their clinical study population into genotype 1 and non-genotype 1. Based on the analysis of genotype response to therapy the sponsor suggested that the non-genotype 1 may respond better than the genotype 1 (see clinical and statistical reviews). With regard to response rates as a function of virus load, subjects with lower levels of HCV RNA appear to respond slightly better than those with higher levels of HCV

[16] Ginocchio, C. C. J. Clin. Microbiol. (1997) 35: 2886-2893

[17] Griffith, B. P. J. Clin. Microbiol. (1997) 35: 3288-3291

[18] Martinot-Peignoux, M. et al., Hepatology (1995) 22: 1050-1056

RNA (see clinical and statistical reviews). However, a similar study[19] comparing the effect of interferon alone with interferon plus ribavirin in previously untreated patients reported that sustained response rates in the interferon and ribavirin combination was independent of virus load.

Determination of HCV RNA in clinical samples by an experimental RT-PCR protocol adopted and conducted by the sponsor exclusively at their facilities by design (and in effect) was a qualitative procedure capable of scoring HCV positive samples from HCV negative samples. It was not possible to determine the assay's lower limit of detection of HCV RNA copies with the meager data supplied. Furthermore, the extent of false positivity and false negativity of the assay was unknown as the sponsor had not addressed this aspect of the assay. Thus, while the experimental RT-PCR procedure used in the clinical studies was capable of detecting HCV RNA, it was incapable of defining a lower limit for detection of HCV RNA copy number. Therefore, in the label where appropriate the HCV RNA response was indicated as being below the limit of detection using a research based RT-PCR assay without specifying a number for the HCV RNA copies.

Draft Microbiology label: ***Mechanism of action***: *Interferon alfa-2b, recombinant/ ribavirin.* The mechanism of inhibition of hepatitis C virus (HCV) RNA by combination therapy with INTRON A and REBETOL has not been established.

RECOMMENDATION: The microbiology portion of the draft label as currently written is acceptable. With respect to microbiology this NDA is approved.

/S/

Narayana Battula, Ph.D.
Microbiologist

[19] Reichard, O. et al., Lancet (1998) **351**: 83-87